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Effect of Processing Technologies on the Allergenicity of Food Products

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ABSTRACT

Heat treatment has been used since ancient times for food processing, firstly to ensure the safety of food and its storage, but also to transform its characteristics (in its raw form) and obtain new textures, flavors, or novel foods. However, the transformation experienced by food components when heated, or processed, can dramatically affect the allergenicity of food, either reducing, or increasing it. To date, most of the articles published dealing with the changes in the potential allergenicity of food are focused on heat treatment and the Maillard reaction. However, it is also important to give prominence to other group of new technologies developed nowadays, such as high-pressure processing, microwaves and food irradiation. These techniques are not likely to replace traditional processing methods, but they are becoming attractive for the food industry due to different reasons, and it is expected in the near future to have different products on the market processed with these new technologies at an affordable cost. Moreover, other biochemical modifications, particularly enzymatic cross-linking of proteins, have attracted wide-spread attention and will be considered as well in this review, because of its great opportunities to induce protein modification and thus affect food allergenicity. Together with the effect of processing of food allergens, this review will place special attention on gastroduodenal digestion of processed allergens, which directly affects their allergenicity.

Keywords: digestibility, heating, Maillard reaction, high-pressure, microwaves, irradiation, enzymatic cross linking

1. *INTRODUCTION*

The term food allergy is used to describe an adverse immune response to foods. Food allergic disorders include acute, possibly life-threatening allergic reactions, as well as chronic debilitating diseases such as atopic dermatitis or eosinophilic gastroenteropathies (Sicherer and Sampson, 2009). Although an allergy could be triggered by virtually any food, the “major allergens” responsible for most significant reactions include milk, egg, peanut, tree nuts, shellfish, fish, wheat and soy. Most of these major food allergens share a number of common features such as being water-soluble glycoproteins of 10 to 70 kDa in size, and relatively stable to heat, acid and proteases.

Food allergy rates vary by age, local diet and geographical area among other factors. Studies in the US have indicated an 18% increase in childhood food allergy from 1997 to 2007. Some childhood allergies are expected to be outgrown by age 5, however there is an estimated 3.9% of children that are currently being affected (Branum and Lukacs, 2009). Food allergy is the most common cause of anaphylaxis as reported by the emergency departments of the US and UK, which have registered an increase of more than 3-fold in the number of hospitalizations for food induced anaphylaxis (Sicherer et al., 2010). The current management of food allergy is limited to strict avoidance, nutritional counseling, and emergency treatment of adverse reactions. Although attempts to desensitize patients date back more than 100 years, there are not accepted therapies proved to accelerate the development of oral tolerance or to provide effective protection from unintentional exposures.

Different approaches have been attempted to reduce allergenicity of food as it is the case of genetically engineered foods (Herman, 2003) or extensively hydrolyzed formulas (Crittenden and Bennett, 2005) since food processing, but one aspect that definitely affects proteins allergenicity. Processing may destroy existing epitopes on a protein or may generate new ones (neoallergen formation) as a result of changes involving protein conformation (Sathe et al., 2005). On whole, conformational epitopes, which are dependent on the tertiary organization of the protein, are expected to be more susceptible to processing-induced destruction than sequential epitopes, the latter being more likely to be altered through enzymatic hydrolysis. Alternatively, sequential epitopes may be chemically modified during food processing or be intentionally changed by introducing mutations through genetic engineering. Generally, decreased allergenicity can be reached through destruction, modification and masking of the epitopes. Depending on the processing method and the applied conditions, the results can be very different (Sathe and Sharma, 2009; Maleki, 2004), even different processing conditions of the same food may lead to different effects (Nowak-Wegrzyn and Fiocchi, 2009). As an example, in Sicherer and Sampson (2007) is showed that emulsification of peanut (peanut butter) increases allergenicity through an adjuvant effect while 70-80% of young children allergic to egg can tolerate baked (heat-denatured) forms of the protein but not the unbaked forms (Lemon-Mule et al., 2008).

The effect of processing on the gastrointestinal digestion of food allergens is another feature that deserves to be considered in this review. Although the mechanisms of food allergy remain unclear, it is thought that an allergen is able to survive the harsh acidic and proteolytic environment of the stomach or tolerate the presence of surfactants in order to reach the gut

associated lymphoid tissue (Polovic et al., 2007). Many of the food allergens are stable proteins that are very resistant to digestion by gastrointestinal enzymes (Fu et al., 2002; Moreno, 2007) or can be digested into high molecular weight peptide fragments which retain IgE binding and T-cell stimulating capacities (Lehman et al., 2006). In this regard, food processing can have an influence by either making the allergens more susceptible to gastrointestinal enzymes or by producing hypoallergenic hydrolysates unable to elicit immune responses.

This paper reviews current knowledge of how food processing modifies the allergenic potential of food. For the purpose of this review, in those technological treatments in which a high amount of information is available, the allergens will be individually discussed.

2. HEAT TREATMENT

Thermal treatments are usually applied to food to keep or improve microbiological quality or to process the food. Heat treatment of food proteins may produce different modifications including denaturation, hydrolysis of peptide bonds, aggregation by non-covalent and disulphide bonds and reactions with other food molecules such as lipids and carbohydrates. As a consequence of this ample array of reactions, thermal processing will have a strong influence on the proteins allergenicity by either reducing (loss of epitopes) or enhancing (exposure of epitopes or generation of new ones) it (Wal, 2003).

2.1 Milk

Heating is one of the most common technological treatments applied during milk processing with different effects on protein IgE binding depending on the heat conditions. Mild heat

treatments are not enough to reduce the allergenicity of milk as has been shown for pasteurized milk, which is able to elicit allergic responses in milk allergic patients (Host and Samuelsson, 1988; Norgaard and Bindslevjensen, 1992), or even boiled milk for 2 and 5 min, which did not make any significant difference in allergenicity as assessed by skin prick test (Norgaard et al., 1996). Besides the heat treatment applied, these differences are also explained by the protein content, and the way that antigens are presented to patients (i.e. the matrix can react with the antigens). As an example a clinical study showed that 75% of children with milk allergy tolerated a muffin baked at 177°C for 30 min and a waffle cooked at 260°C for 2 min (around 1.3 g of milk protein) (Nowak-Wegrzyn et al., 2008).

Focusing on some of the main milk allergens, patients are mainly sensitized to β -lactoglobulin that is susceptible to heat denaturation, and to α , β and κ -casein that are naturally unfolded proteins in which their allergenicity is not affected by heat treatment, but it may alter their resistance to the digestive process. It has been shown that β -lactoglobulin IgE-binding and resistance to digestive enzymes are reduced under heat treatment in a dose-dependent manner (Ehn et al., 2004; Taheri-Kafrani et al., 2009; Morisawa et al., 2009). Regarding α -casein, in an interesting study where human basophils were passively sensitized using sera from milk allergic patients it was shown that IgE-binding properties were not affected by thermal treatment and the protein maintained a high susceptibility to proteolysis (Morisawa et al., 2009) although Dupont et al. (2010) observed the opposite effect. They reported a higher resistance to digestion when digested following an infant *in vitro* model mainly characterized by a higher pH during gastric digestion. These contradictory results are likely attributed to a different gastric stage of heated α -caseins with a higher gastric pH. Regarding κ - and α s2-casein, their ability to generate

aggregates with whey proteins under severe heating conditions (95°C for 10 min) has been described (Guyomarch et al., 2003). Those aggregates could protect them from digestive enzymes, thus increasing the sensitizing capacities of the proteins (Roth-Walter et al., 2008). The effect of different heat treatments on IgE-binding and digestibility of milk or milk proteins is summarized in **Table 1**.

2.2 Egg

The effect of heat treatment on egg has been largely studied, especially on the albumen where the main egg allergens are contained (Cooke and Sampson, 1997; Mine and Zhang, 2002). Urisu et al. (1997) conducted a clinical study where egg allergic patients were orally challenged to heated egg white and heated ovomucoid-depleted egg white (90°C for 60 min). The heated egg white was tolerated for half of the patients while the heated ovomucoid-depleted egg white was tolerated for 95% of the patients. This study revealed the importance of ovomucoid in the pathogenesis of egg-allergy as a thermoresistant allergen, in comparison to heat-unstable egg white allergens such as ovalbumin, ovotransferrin and lysozyme. More recently, and in agreement with those results, Urisu et al. (2008) and Jimenez-Saiz et al. (2011a) obtained positive results with the use of heated ovomucoid-depleted egg white in an oral immunotherapy protocol with egg allergic children and egg white sensitized mice respectively. In addition, Jurado-Palomo et al. (2010) showed that 66% of egg-allergic children orally challenged to pasteurized albumen were able to tolerate it.

The effect of thermal treatment on whole egg has also been studied. Des Roches et al. (2006) carried out a clinical study which stated that 73% of egg allergic children beyond the age of 5

tolerated cooked egg as an ingredient of a cake. Lemon-Mulé et al. (2008) showed similar results in a clinical study: around 70% of the egg allergic patients challenged to heated egg baked in a wheat matrix (in the form of a muffin and a waffle) were tolerant. However, it is noteworthy that heat treatment and baking are not fully comparable since in the case of baking products such as cake, muffin or waffle, the food matrix might play a key role in the tolerance induction. For example, the formation of complexes between ovomucoid and gluten is one of the mechanisms suggested as responsible of the aggregation and insolubilization of the allergens (Benhamou et al., 2010).

Another way by which heat treatments could affect the immunogenic properties of egg allergens is by modifying their susceptibility to gastrointestinal digestion. Takagi et al. (2003) studied the effect of heating (100°C for 5 min) on ovalbumin *in vitro* digestion and found that thermal treatment markedly increased the digestibility of ovalbumin in both simulated gastric and intestinal fluid. In agreement, Jiménez-Saiz et al. (2011b) demonstrated that heat treated (95°C for 15 min) ovalbumin was more susceptible to digestion while it did not affect ovomucoid. It has been recently postulated that fragments of heated ovalbumin lead to the shift from Th2 to Th1-type responses as compared to native ovalbumin, as seen by Golias et al. (2012) in a mouse model. Furthermore, Martos et al. (2011) have shown that heating of intact ovalbumin and ovomucoid might prevent transport across human intestinal epithelial cell in a form capable of triggering basophil and T-cell activation.

2.3 Peanut

The effect of heat treatment on peanut allergenicity has been extensively studied over time and currently it is still a topic of research (Cong et al., 2007; Hu et al., 2010; Maleki et al., 2010). Early studies showed that different thermal treatments did not change peanuts allergenicity (Nordlee et al., 1981; Burks et al., 1992). In an extensive research, Koppelman et al. (1999) studied the effect of different heat treatments on Ara h 1, the major peanut allergen, and reported that Ara h 1 kept similar IgE-binding properties to that of the unheated form since the protein structure did not change under heating conditions. Later on, Mondoulet et al. (2005) analyzed the impact of roasting or boiling on whole peanut proteins and purified Ara h1 and Ara h 2. Surprisingly, the IgE responses to raw and roasted peanut extracts were not significantly different, whereas those to boiled peanut extracts were approximately 1.5-2-fold lower, which was attributed to a loss of low-molecular-weight proteins or peptide fragments from kernels and their transfer by solubilisation into the cooking water. Boiling of Ara h 1 also results in the formation of aggregates with reduced allergenicity (Blanc et al., 2011). Interestingly, Ara h1 and Ara h 2 purified from roasted peanut extracts showed higher IgE binding than those purified from raw and boiled peanut extracts although this difference was no longer apparent with the whole food. The fact that roasting increases allergenicity of Ara h1 and Ara h 2 has been attributed to either neo-epitopes formation by Maillard Reaction (Maleki et al., 2000), or exposure of hidden ones. Also the increased allergenicity of peanuts after roasting might be due to a higher output of allergens extraction in roasted peanuts (Pomes et al., 2006). Recently, it has been reported that autoclaving at 2.56 atm for 30 min produces a significant decrease of IgE-binding of peanut allergens due to changes in their secondary structure (Cabanillas et al., 2012).

A number of studies have been conducted on peanut allergens digestion (Burks et al., 1992; Van Voxel et al., 2008a; Koppelman et al., 2010). In general, Ara h 2 has been reported to be more stable to peptic digestion compared with Ara h 1 (Koppelman et al., 2010). Van Voxel et al. (2008a) showed that thermal treatment (100°C for 10 min) did not change digestion stability of Ara h 1 in oligomeric or trimeric form. On the other hand, it has been shown that some heat treatments, such as roasting, are able to increase the trypsin inhibitory activity of Ara h 2 approximately 3.5-fold, which results in a lower hydrolysis degree of Ara h 1 when both are digested together after roasting (Maleki et al., 2003). It has been reported that solubility changes on peanut proteins induced by heat treatment also might contribute to their allergenic potential. Indeed, Kopper et al. (2005) found that progressive roasting of peanuts results in a significant solubility decrease that makes their digestion difficult, but when proteins are solubilised in the gastric environment, by acid pH, they keep a high IgE-binding capacity, hence the low soluble state of peanut proteins under roasting could help peanut allergens to reach the gut associated lymphoid tissue.

2.4 Tree nuts

Tree nuts are a large and complex group of allergens where thermal processing effect in terms of antigenicity, allergenicity and digestibility has been researched reaching a heterogeneous outcome (**Table 2**). Walnuts have been reported to be highly resistant to heat treatments, keeping their antigenicity after combined treatments of γ -irradiation and heating (Su et al., 2004). Also, hazelnut (Muller et al., 2000; Schocker et al., 2000), almonds (Venkatachalam et al., 2002), pecans (Venkatachalam et al., 2006) and cashews (Venkatachalam

et al., 2008) have revealed the same results. Van Boxtel et al. (2008b) demonstrated the high thermo resistance of the major allergen of the Brazil nut (Ber e 1) when they heated Ber e 1 extracts at different pHs (2, 5, 7) and temperatures up to 120°C. Ber e 1 was shown to denature at 110°C and pH 5-7 but it also denatured at 80°C in a very acid medium. This is likely due to the conformational changes of the protein caused by pH that made Ber e 1 more susceptible to heat-induced changes. Accordingly, Moreno et al. (2005) published that heat processing (100°C for 20 min) of Ber e 1 does not change its resistance to gastrointestinal digestion, which suggests that heated Ber e 1 retains its allergenicity. Rouge et al. (2011) also reported a strong resistance to heat denaturation and hydrolysis by digestive proteases for cashew nut allergens. Finally, a research conducted by Noorbaksh et al. (2010) investigated the effect of processing on pistachio IgE-binding. Interestingly, the steam-roasting process seemed to reduce IgE-binding of pistachio more than dry roasting did, whereas the thermal processing increased the resistance to digestion of the pistachio proteins.

2.5 Wheat

Wheat is usually consumed as a food ingredient in bread or pasta, products that have already undergone a heat treatment. The way thermal treatments affect wheat allergenicity, digestibility and solubility have been investigated in depth. Baking does not significantly affect IgE-binding of wheat allergens as part of bread (crust and crumb) compared to the dough, only the IgE-binding of heat-labile α -amylase inhibitor protein family is dramatically reduced (Pasini et al., 2001; Simonato et al., 2001a). Moreover, baking seems to protect wheat allergens from digestive enzymes by strong molecular linkages different than disulfide bonds or hydrophobic interactions,

especially in the crust where higher temperatures are reached, resulting in a higher IgE-binding of duodenal digests compared to that of the dough (Simonato et al., 2001a). On the contrary, the IgE-binding potential of heated wheat flour was reduced after heating (Varjonen et al., 1996). The ability of wheat proteins to bind IgE, as part of cooked commercial pasta has been reported to be reduced, but not abolished, after *in vitro* digestion (De Zorzi et al., 2007). Actually, cooked pasta is unsafe for allergic patients based on the gastrointestinal symptoms developed in a clinical study where the patients were challenged with cooked pasta (Simonato et al., 2001b). A drastic effect of heat treatment, clearly seen in wheat proteins, is a loss of solubility because of the heat-induced structural changes. It has been published that wheat proteins from boiled pasta became insoluble up to 23% and 39% when dried at 110 and 180°C respectively (De Zorzi et al., 2007). The formation of protein aggregates, after extensive heating, with enhanced resistance to digestion might favour the presence of allergenic structures in the gut and the risk of eliciting an immune response.

2.6 Soybean

Heat treatment has been shown to affect soybean allergenicity differently (Lee et al., 2009; Mueller et al., 1998). The first observations indicated that heating did not affect IgE-binding of the crude soybean fraction (Burks et al., 1991) although there was an increase of the 2S fraction IgE-binding properties when heating at 80°C. The remaining fractions had a great reduction in their IgE-binding activity compared to that of the crude protein (Shibasaki et al., 1980). Similarly, an increase in the antigenicity of P34, the immunodominant soybean allergen, was reported after 5 min of boiling, probably because of an exposure of linear epitopes hidden in the

secondary structure; as the boiling continued, antigenicity was slowly reduced until no binding was detected (Wilson et al., 2008). Codina et al. (1998) saw an increase of IgE-binding when soybean hull proteins were heated, which was attributed to two novel allergenic determinants of low molecular weight (10 and 15.3 kDa).

Regarding digestion, Takagi et al. (2003) studied the effect of preheating (100°C for 5 min) on the digestibility of soybean trypsin inhibitor but they did not notice any effect in gastric and duodenal digestion, which suggests that the allergenicity of the digests from heated soybean trypsin inhibitor is similar than to those of the unheated form.

2.7 Fish

The effect of heat treatment on fish allergens has been widely researched but it is still under investigation because of the different outcomes and the elevated number of species. Although it is accepted that parvalbumins are heat-stable and resistant to enzymatic degradation (Wild and Leher, 2005), their stability has been questioned through time. Heat treatments such as steam sterilization (Chopin et al., 2000), boiling or frying (Chatterjee et al., 2006) have been reported to reduce IgE-binding properties of the fish allergens (Table 3). Nevertheless, smoking is able to induce aggregates formation with strong IgE-binding properties (Sletten et al., 2010). In the same line, Griesmeier et al. (2010) reported that Lep w 1, the major allergen of whiff (*Lepidorhombus whiffiagonis*), under heating (100°C for 10 min) formed dimeric aggregates which were resistant to digestion and more allergenic than the uncooked form. In studies conducted with 110 human sera Chatterjee et al. (2006) and Mondal et al. (2007) found that IgE-binding of heated mackerel (*Rastrelliger kanagurta*) or bethki (*Lates calcarifer*) (boiling or frying) was similar to or higher

than that of the unheated extract. Furthermore, heated bethki and mackerel showed higher resistance to digestion and both retained strong IgE-binding activity after gastric digestion in comparison to the uncooked extracts. It is important to highlight the presence of *Anisakis simplex* (a parasitic nematode) in fish since it is an extra source of allergens. Heat treatments reaching 70°C are usually recommended to kill *Anisakis simplex*, however, even if this treatment is appropriate from a microbiological perspective, it does not seem to be sufficient from an immunological point of view because *Anisakis* allergens keep their antigenicity after extensive heating at 94°C for 60 min (Vidacek et al., 2010) as well as their IgE-binding after boiling for 30 min (Caballero and Moneo, 2004). Traditionally the use of heat treatment to inactivate *Anisakis simplex* is suggested (Bouree et al., 1995), but other authors support the use of freezing (-20°C) since it causes death of the parasite and it seems to be safer, in terms of allergenicity (Alonso et al., 1999; García et al., 2001; Trujillo et al., 2002).

2.8 Shellfish

Thermal treatment seems to exert a less variable effect on shellfish allergens compared to other foods although it is worth saying that the majority of the published studies only used boiling as the heating method, reducing the variability introduced when different treatments are used. All the studies agree in reporting the ability of tropomyosin to maintain a great allergenic potential even after heat-denaturation (Carnes et al., 2007; Liu et al., 2010; Lopata et al., 2010; Yadzir et al., 2010) (Table 4). Tropomyosin shows immunological reactivity after high temperature even after being digested by trypsin or chymotrypsin (Yu et al., 2011). Furthermore, shellfish is usually consumed boiled or grilled, which explains that patients are sensitized to the

allergens resulting from heating. In fact, Martin-Garcia et al. (2007) described the case of a woman with selective hypersensitivity to boiled razor shells.

The stability of some shellfish allergens after heating to digestive enzymes has also been studied. Lee and Park (2004) reported that thermal treatment did not affect IgE-binding of whelk allergens nor its resistance to digestion, which was similar to that of the unheated whelk, although both were degraded by digestive enzymes. On the contrary, Shimakura et al. (2005) found that allergenicity of different types of shrimp and crab extracts boiled for 15 min was almost completely abrogated after digestion. Lately, a new allergen, the octopus arginine kinase has been described, showing to be unstable to thermal treatment, which results in a reduced IgE binding activity after heat processing (Shen et al., 2012).

3. *MAILLARD REACTION*

The Maillard reaction (MR) is a non-enzymatic reaction between amino acids of proteins and non-reducing sugars which occurs in foods during heat treatment or after long-term storage (Nursten, 1981). In this reaction, the free amino groups of proteins undergo nonenzymatic glycosylation to the formation of Schiff bases, which suffer a rearrangement to form Amadori products. Through a series of rearrangements, cyclizations, and dehydrations, the Amadori products form structurally diverse compounds known as advanced MR products or advanced glycation end products (Booth et al., 1997). The interaction with sugars may modify the tertiary structure of the proteins and thus modify their conformational epitopes, creating novel IgE binding sites, masking the allergenic structure or exposing previously unavailable sites (Maleki and Hurlburt, 2004). Furthermore, advanced glycation products derived from food allergens can

alter dendritic cell function and subsequently affect on allergen-specific T cells (Ilchmann et al., 2010).

3.1 Milk

Regarding the binding of milk proteins with different carbohydrates by MR, most of the experiments have been carried out with β -lactoglobulin. For example, β -lactoglobulin has been conjugated with carboxymethyl dextran, alginic acid and phosphoryl oligosaccharides and it has been found that the conjugates have a significantly lower immunogenicity than that of native protein (Hattori et al., 2000 and 2004; Yoshida et al., 2005). In the case of carboxymethyl dextran, the higher its molecular weight, the more potent the effect. Authors hypothesized that covering the epitopes by the carboxymethyl dextran chain might be the main reason for the reduced immunogenicity observed (Kobayashi et al., 2001; Kobayashi et al., 2003). Conjugation of β -lactoglobulin with ribose and lactose has also been investigated. Ribosylated β -lactoglobulin showed a considerable increase of the IC₅₀ indicating that the recognition of β -lactoglobulin by IgE from milk allergic patients is impaired by glycation with ribose. The authors found that a high degree of glycation resulted in a clear “masking” effect on the recognition of epitopes. Regarding lactose conjugation, no significant differences were found between the control and the lactosylated form (Taheri-Kafrani et al., 2009). Interestingly, a promising model regulated by three independent variables for optimal reaction conditions to obtain a lower antigenicity of β -lactoglobulin (Bu et al., 2010) and α -lactalbumin (Bu et al., 2009) with glucose has been established. The optimum values of the variables to yield minimum levels of the antigenicity were as follows: whey protein isolate/glucose weight ratio, 2.59 and 5.96, temperature, 51.88 and

52.80 °C; and time, 75.7 and 78 h for β -lactoglobulin and α -lactalbumin respectively. Under the above glycation reaction conditions, the antigenicity of β -lactoglobulin and α -lactalbumin were decreased by 99.68% and 95.22% respectively by the MR compared to native whey protein isolate.

As a result of the effect of glycation on the conformation and physico-chemical properties of the proteins, their susceptibility to proteolysis is altered (Moreno et al., 2008) and subsequently it is expected that their intestinal absorption is also different modifying their allergenic properties. In this regard, the allergenic potential of the hydrolysates of β -lactoglobulin MR complexes with galactose, tagatose and dextran of 10 kDa or 20 kDa after simulated gastrointestinal digestion have been determined (Corzo-Martínez et al., 2010). Whereas a high degree of glycation with galactose and tagatose impaired the β -lactoglobulin proteolysis and, consequently, significantly increased the IgE binding of the hydrolysates, the formation of protein aggregates during the advanced stages of the MR had a masking effect on the β -lactoglobulin epitopes, counteracting the negative effect of the lower digestibility of glycated protein on its allergenicity. Indeed, no significant differences were found in the serum IgE binding of hydrolysates between control and glycated protein. In the case of the conjugates with dextran, the proteolytic susceptibility of β -lactoglobulin also decreased, but in this case the steric hindrance exerted by the molecules of dextran attached to β -lactoglobulin could also contribute to the lower reactivity of digestive enzymes toward this protein, then glycation with dextran increased the response against IgE antibodies as compared to that of the unglycated protein digested.

3.2 Egg

Studies on the influence of the MR have been carried out on ovalbumin and ovomucoid with glucose. Jiménez-Saiz et al. (2011b) reported a lower IgE binding to glycosylated ovalbumin but a higher IgE binding to glycosylated ovomucoid. Ilchmann et al. (2010) evaluated the ability of glycosylated ovalbumin to activate ovalbumin-specific T-cells. Their results showed that compared with the native protein, the glycosylated ovalbumin enhanced the activation of ovalbumin-specific CD4⁺ T cells when cocultured with myeloid dendritic cells. Similar results have been described by Hilmenyuk et al. (2009).

The influence of MR on the hydrolysis of the egg white major allergens has also been aim of research. Ovalbumin digestibility is impaired by glycosylation with glucose, while that of the ovomucoid is not affected. The changes observed in the digestibility of ovalbumin counteracted the initial reduction of IgE binding capacity induced by MR so that the gastroduodenal digests of glycosylated ovalbumin showed similar reactivity to the digests of the native protein (Jiménez-Saiz et al., 2011b).

3.3 Peanut

The high levels of reducing sugars contained in peanut make the occurrence of MR very likely (Chung and Champagne, 1999). However, the results described on the allergenicity of the MR products are not completely clear. Clare et al. (2007) showed that glycoprotein conjugates, created by covalent linkage between peanut protein fractions and monosaccharides, exhibited similar IgE binding activity, compared to control solutions. Moreover, the peanut allergens, Ara

h 1 and Ara h 2, have been shown to increase their allergenicity with the MR in some studies (Chung and Champagne, 2001; Gruber et al., 2005). Vissers et al. (2011) have reported that Ara h 2 and 6 purified from roasted peanut retained the structure and IgE reactivity/functionality of the native protein which may explain the allergenic potency of peanuts. Maleki et al. (2000) demonstrated that roasting peanuts can increase IgE binding capacity of Ara h 1 and Ara h 2 up to 90 times, decreasing the solubility and digestibility of proteins due to structural alterations including denaturation, aggregation and crosslinking, responsible for the creation of new IgE-binding epitopes, and increase the exposure of existing ones. Nevertheless, they hypothesized that the increase in the IgE-binding properties of particular peanut allergens subjected to MR is not enough to account for the large increase in the IgE binding by roasted peanut extracts, so multiple biochemical reactions during processing and the collective modifications to all peanut proteins could be accounting for the increased IgE binding.

Regarding Ara h 6, although it is highly homologous to a large section of Ara h 2, it behaves differently under glycation conditions. Contrary to Ara h 2, glycated Ara h 6 is less immunoreactive than native Ara h 6 (Sotolongo et al., 2006). It could be attributed to the function of Ara h 2 as an inhibitor of trypsin activity that is increased 3.6 times after roasting, which may reflect increased allergenicity (Maleki et al., 2003). Moreover, these authors found that Ara h 2 protected Ara h 1, a second major peanut allergen, from degradation by trypsin.

3.4 Wheat

A few research groups have focused on the effect of MR on wheat proteins. Nakamura et al. (2008) linked covalently the major allergenic protein of buckwheat, Fag e 1, with the food-grade polysaccharides arabinogalactan and xyloglucan through controlled dry-heating at 60 °C and 65% of relative humidity, obtaining a drastic reduction in the reactivity against human sera from buckwheat-allergy subjects. Moreover, Nakamura et al. (2007) revealed that the smaller xyloglucan, was more effective for the purpose of reducing the allergenicity than the larger arabinogalactan likely due to a more efficient binding of xyloglucan, on regions where epitopes were placed or were affected, than arabinogalactan.

3.5 Soybeans

MR reaction has also been proposed as a way to reduce the allergenicity of soy proteins. Babiker et al. (1998) showed that conjugation with galactomannan reduced the allergenicity of the major allergen of soybean, P34. Furthermore, Usui et al. (2004) and Van de Lagemaat et al. (2007) demonstrated that this decrease was more pronounced using chitosan for conjugation. Other carbohydrates employed successfully in the reduction of the antigenicity of soy protein by glycation are fructose and fructooligosaccharides (Van de Lagemaat et al., 2007; Bu et al., 2009 and 2010).

3.6 Shellfish

The effect of MR on the allergenicity of tropomyosin was investigated by Nakamura et al. (2005). The IgE-binding ability of scallop tropomyosin increased significantly with the progress of the reaction with glucose, ribose, and maltose, but not with maltotriose. Additionally, it was confirmed that the enhancement of the allergenicity of tropomyosin -sugars is not related to the protein surface charge, indicating that the allergenic variation would be caused by the protein structural change occurring at the early stage of the MR. These results were completely different from those obtained with squid tropomyosin. When tropomyosin reacted with ribose, its human-specific IgE-binding ability decreased markedly (Nakamura et al., 2006). A possible explanation given by the authors is that there is only 69.7% of amino acid sequence homology between squid and scallop tropomyosin and, therefore, there may be different epitope sites between both tropomyosins. The distinct structural changes induced by the MR with ribose can also contribute to such differences because 21% of lysine residues exist in different positions between them.

4. HIGH HYDROSTATIC PRESSURE

High hydrostatic pressure (HHP) has been employed in the food industry to inactivate microorganisms and enzymes and improve product texture by protein alteration and denaturation (Mozhaev et al., 1996). HHP can lead to a partial or complete inactivation of the biological function of the protein depending on pressure, temperature and chemical conditions. The effects have been attributed to changes in noncovalent bonds, such as electrostatic and hydrophobic

interactions, which cause different reversible and irreversible changes on the levels of quaternary, tertiary, and secondary structures (Meyer-Pittroff et al., 2007).

It is known that secondary and tertiary structure of food allergens is crucial to their allergenic potential. The way in which allergens are folded potentially affects digestion in the gastrointestinal tract and therefore the presence of allergenic peptides in the gut and recognition by serum IgE. Thus, it has been theorized that HHP can change allergen reactivity by altering the structure of food allergens. A good example corresponds to those experiments performed by Yamamoto et al. (2010) in which they demonstrated that HHP treatments above 100 MPa led to a change in the tertiary structure of bovine γ -globulin, one of the major beef allergens. As a consequence of this structural modification they reported both a significant decrease in the IgE specific binding and a diminished production of β -hexosaminidase by mast cells when using HHP-treated γ -globulin as an allergen. The same group also found a significant decrease in the allergenicity of wheat α -amylase inhibitor when treated with HHP above 200 MPa although in this occasion they did not find clear structural changes in the tertiary structure, probably because most of the epitopes do not contain tyrosine residues, and hence, they were not detectable by the technology employed. Unfortunately, HHP technology does not affect all allergens similarly. It has been demonstrated that HHP treatments ranging from 300-600 MPa are unable to modify the allergenic properties of carrot (Heroldova et al., 2009), peanut (Johnson et al., 2010) milk whey (Kleber et al., 2007; Chicón et al., 2008a) silver carp (Liu and Xue, 2010), Anisakis (Vidacek et al., 2009) celery (Houska et al., 2009a, Husband et al., 2011), apple (Fernández et al., 2009; Houska et al., 2009b, Husband et al., 2011) and tomato (Germini et al., 2007) main allergens.

As it happens with other technologies, the most effective way to reduce allergenicity by HHP is the combination with other processes that may act synergistically. Regarding combination of HHP with heat, a few reports have been published. Hildebrandt et al. (2010) investigated the effects of heat and pressure on the binding of IgE to egg white proteins used as an ingredient in processed meat. They found that although heat treatment and HHP were able to reduce the allergenic potential of the sample individually, the HHP treatment of a sample that was preheated at 70°C resulted in a nearly 2 fold stronger reduction measured by EAST inhibition. Similarly, Husband et al. (2011) recently reported the strong resistance to thermal processing of one of the main allergens from apple, Mal d 3. However, the combination of HHP and temperature (700 MPa, 115°C) significantly reduced the immunoreactivity of this allergen. Surprisingly, Mal d 1, another main allergen from apple, retained the allergenicity after being subjected to both treatments together (Fernández et al., 2009). Without any doubt, the most studied approach in combination with HHP to reduce the antigenicity of food proteins is the enzymatic hydrolysis. Under HHP, some allergens such as β -lactoglobulin form transient conformers, with ample regions of the hydrophobic core unfolded and new target bonds exposed to enzymatic hydrolysis, as compared with the compact native protein (Chicón et al., 2006a). This allows obtaining protein hydrolysates with allergenic and functional characteristics different to those obtained at atmospheric pressure. López-Expósito et al. (2008) hydrolyzed egg white ovalbumin with pepsin under 400 MPa. All the intact protein was rapidly digested, leading to the production of hydrolysates with lower antigenicity than those produced in hours at atmospheric pressure. Nonetheless, the hydrolysates retained some residual IgE binding properties as a result of the accumulation of large and hydrophobic peptides during the initial stages of the digestion.

Likewise, when β -lactoglobulin was incubated with pepsin under the same HHP conditions similar results were obtained. On the other hand, in these experiments extending the hydrolysis time produced a considerable reduction of the IgE binding properties to almost negligible (Chicón et al., 2008b). The same group also applied this treatment (hydrolysis with pepsin under 400 MPa) to the production of whey protein digests with reduced immunogenicity. In addition to the low immunogenicity, the hydrolysates showed good functional properties, such as heat stability and emulsifying activity, which makes them good candidates to prepare a stable hypoallergenic formulation (Chicón et al., 2009). It is important to highlight that not only pepsin has been employed to reduce the immunogenicity of β -lactoglobulin. Other enzymes such as chymotrypsin (Bonomi et al., 2003; Chicón et al., 2006a), trypsin (Peñas et al., 2006a, Chicón et al., 2006b), alcalase, neutrase and papain (Peñas et al., 2006b) have also been used successfully to achieve results equivalent to those obtained with pepsin. Recently, the first *in vivo* study on the ability of HHP β -lactoglobulin hydrolysates to elicit anaphylactic reactions has been published (López-Expósito et al., 2012). It was shown that peptic and chymotryptic β -lactoglobulin hydrolysates carried out at 400 MPa during 5 and 20 min respectively had an abrogated allergenicity. This was demonstrated by the absence of symptoms and a decrease in body temperature in a mouse model of food allergy. This study revealed that although the HHP hydrolysates had some *in vitro* residual IgE-binding, they had lost their ability to cross-link 2 human IgE antibodies to induce mast cell degranulation, thus indicating that most of the peptides formed retain just one relevant IgE-binding epitope.

5. MICROWAVES

Microwave heating is accomplished by both absorption of the microwave energy by rotation of the dipolar water molecules and by the translation of the ionic components of food. Microwaves are shown to affect the kinetics of conformational changes of proteins and to accelerate their denaturation (Bohr and Bohr, 2000). Those changes in the native structure of proteins might have a profound influence on their sensitizing properties. Despite some attempts have been done in using microwaves as a technology to reduce the immunoreactivity of food allergens (Venkatachalam et al., 2008), research shows that microwaves are not sufficient in themselves to reduce or eliminate the antigenicity of proteins and should be combined with other processes such as an enzymatic hydrolysis or a decrease in pH to make them more effective (Kaddouri et al., 2006; Leszczynska et al., 2003; Venkatachalam et al., 2002). Izquierdo et al. (2008) observed a decrease in the immunoreactivity when combining microwave heating with pronase, papain or alcalase to obtain enzymatic hydrolysates from whey protein concentrate. The reduced immunoreactivity obtained was explained by an increased accessibility of the enzymes to epitopes that were hidden in the protein structure before applying the microwaves treatment. A novel study reported extensive hydrolysis of the microwave-treated β -lactoglobulin and whey protein concentrate with trypsin, chymotrypsin, and the mixture of trypsin with chymotrypsin, but any impact on the IgE binding of the products obtained in all the studied conditions (El Merchefi et al., 2011). Also, it has been shown that when the pH value is decreased to 4.6, microwave heating of whey proteins at 300 W results in a loss of immunoreactivity up to 47% while at the natural milk pH the loss of antigenicity is moderate (29.32%) (Grar et al., 2009).

6. IRRADIATION

Food irradiation is a treatment consistent in exposing foods to ionizing radiation of usually high-energy electrons or electromagnetic waves (x , γ or UV rays). Food irradiation is used primarily as a preservation method, but it also has potential as a technology to produce specific changes in the food components. Proteins exposure to radiation produces denaturation due to diverse radicals generated from radiolysis such as hydroxyl, hydrogen or hydroperoxyl. In addition, radiation induces fragmentation and aggregation of proteins through strong interactions such as electrostatic or disulfide bonds (Davies and Delsignore, 1987). Irradiation for a short period of exposure (e.g. seconds) may be regarded as nonthermal treatment, whereas extended exposure (e.g. minutes) could cause a pronounced temperature rise and moisture evaporation on foods.

It is known that due to all these structural modifications mentioned, radiation can change the immunogenic properties of the food allergens (Byun et al., 2002).

6.1 Milk

The first recorded data demonstrating the use of irradiation as a technology to reduce the allergenicity of milk dates back from 1961 (Grogan and Crawford, 1961). After that, only a few publications have reported the ability of irradiation to reduce the allergenicity of milk allergens (Lee et al., 2001; Byun et al., 2002). An interesting study by Cho et al. (2010) demonstrated that UV-irradiation of β -lactoglobulin significantly reduces its ability to induce IgE production by

mouse splenocytes. This effect was highly dependent on the irradiation period, reaching the maximum inhibitory effect after 32 h of UV-irradiation exposure. In addition it has been demonstrated that the effect of the radiation on the protein is more effective when the β -lactoglobulin is in solution at a low concentration and high radiation doses (Oliveira et al., 2007; De la Hoz and Netto, 2008).

6.2 Egg

In a novel study, Manzoco and Nicoli (2012) observed a progressive decrease in egg proteins photosensitivity by increasing concentration. Lee et al. (2002a and b) showed that the combination of heat and γ -irradiation (10 kGy) on the egg allergens ovalbumin and ovomucoid was able to efficiently reduce its Ig-binding capacities. The same group evaluated changes of the antigenicity and allergenicity of ovalbumin in white layer cakes containing egg white γ -irradiated. The results indicated that the antigenicity of the ovalbumin augmented. However, the allergenicity decreased a 96% when irradiation of 10 kGy and heat treatment were combined (Lee et al., 2005). Seo et al. (2007) injected irradiated ovalbumin to ovalbumin-sensitized mice to evaluate whether radiation reduced allergenicity. Those mice challenged with irradiated ovalbumin had significantly lower levels of total-IgE than those mice challenged with native ovalbumin. Furthermore, irradiated ovalbumin significantly suppressed production of Th2 cytokines (IL-4 and IL-5) and induced production of Th1 cytokines (IFN- γ and IL-12) in a radiation-dose dependent manner.

6.3 Peanut

Oh et al. (2009) studied the allergenicity and immunogenicity of irradiated peanuts in a mouse model finding that 10 kGy irradiated peanut extract reduced significantly the secretion of IL-4 and increased the production of IFN- γ and IL-10 by peanut-sensitized splenocytes. The effectiveness in reducing IgE binding of peanut extracts and liquid peanut butter after pulsed UV light was also reported by Chung et al. (2008), where untreated samples exhibited and IgE binding 7-fold higher than treated ones.

6.4 Wheat

Contrary to the results obtained with egg allergens and peanut, Leszczynska et al. (2003) demonstrated that irradiation of gliadin and wheat flour with doses between 2.2–12.7 kGy increased their allergenicity. They explained this phenomenon as a consequence of cross-reactions of gliadin with wheat flour components induced by radicals formed during irradiation. These results highlight the influence of the allergen structure on the effects produced by the irradiation. In addition to that, the irradiation dose can also have a remarkable effect on the effectiveness of the treatment. For example, current findings suggest that the allergenic epitopes of wheat-germ agglutinin became less active and antigenic after high-dose radiation (Vaz et al., 2012). Based on the molecular structure, this study examined the structural modification of wheat-germ agglutinin in relation to the range of dose. Results showed a loss of intrinsic activity and the formation of insoluble amorphous aggregates with a lack of native conformational structures after irradiation. The reduction of cytokines typical of allergic reactions, with decreased lymphocytic infiltrate, was observed in the gut of sensitized mice given irradiated

versus native wheat-germ agglutinin. In the same line, Li et al. (2007) showed that irradiated shrimp allergen extracts had a significant decrease in allergenicity when treated at 10 kGy, but lower irradiation doses implied an augment of allergenicity.

7. ENZYMATIC CROSSLINKING

Enzymatic crosslinking of proteins is currently exploited in the food industry as means to stabilize food structure; however it also affects the allergenic properties (Mills and Mackie, 2008). Current applications are based on the enzyme transglutaminase, being able to form an isopeptide bond between glutamine and lysine residues of proteins, although oxidative enzymes such as tyrosinases and laccases are as well currently being investigated as crosslinking agents (Buchert et al., 2007). Nowadays, research on the effect of enzymatic crosslinking on allergenicity of food proteins is very limited. Stanic et al. (2010) have demonstrated the ability of β -casein crosslinked by laccase/cafeic acid to reduce basophil activation in cow's milk-allergic patients. They also demonstrated that crosslinking of β -casein by laccase/cafeic acid or mushroom tyrosinase/cafeic acid are the best treatments to mitigate IgE binding and allergenicity. Moreover, in a basophil-activation assay, the allergenicity of the cross-linked β -lactoglobulin with laccase was shown to decrease in all nine cow's milk-allergic patients (Tantoush et al., 2011). The effect of crosslinking by transglutaminase action was less able to modify the IgE binding epitopes than the presence of small phenolic mediators in the crosslinking process; nonetheless, the polymerization with transglutaminase in the presence of cysteine modified or hidden epitopes, reduced the potential antigenicity of β -lactoglobulin

(Villas-Boas et al., 2010). Furthermore, the polymerization with transglutaminase of chemically denatured β -lactoglobulin facilitated the action of the gastrointestinal enzymes, and its digestion products presented lower antigenic properties than those of the untreated protein (Villas-Boas et al., 2012). In the case of ω -gliadins, transglutaminase treatment did not reduce the allergenic properties of the wheat allergen. The enzymatic cross-linking of peptic digests formed large peptide complexes which bound to IgE antibodies from wheat-allergic patients more intensely than those of the untreated ω -gliadins (Palosuo et al., 2003). On the contrary, it has been described that crosslinking of peanut allergens Ara h 1 and Ara h 2 by peroxidase or polyphenol oxidase/caffeic acid treatment led to a decrease of the levels of both peanut allergens and hence a significantly reduced binding to IgE from peanut-allergic patients (Chung et al., 2004 and 2005). Surprisingly, when peanut allergens were crosslinked with transglutaminase, the effect on the IgE binding properties of the allergens was nonexistent (Clare et al., 2007).

8. CONCLUDING REMARKS

It is well known that processing can change the allergenic capacity of food due to alterations of the epitopes of allergenic proteins (Mills et al., 2009; Paschke, 2009). During processing, proteins can form oligomers, become denatured, degraded, aggregated, cross-linked, fragmented and re-assembled (Maleki and Hurlburt, 2004) and these changes can alter the overall IgE binding profiles of a particular extract (Schmitt et al., 2010). It is critical to keep in mind that the food processing effects observed are highly related to the considered protein and the degree of processing. These important changes that proteins may undergo after food

processing are directly responsible for a higher or lower susceptibility (or resistance) to digestion, which may impact again on the allergenic potential of food proteins since food allergens must be able to cross the intestinal barrier retaining sufficient structural integrity to interact with the gut associated lymphoid tissue (Astwood et al., 1996; Taylor, 2003). For this reason, within the field of food allergy, it is essential to evaluate the digestion resistance of processed proteins, as a part of the matrix in which they are usually consumed, as well as the ability of the fragments generated upon gastro intestinal digestion, ideally *in vivo* or at least *in vitro* by using reliable models that mimic physiological conditions, to retain biologically relevant IgE epitopes.

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Table 1. Effect of heat treatment on IgE binding and digestibility of milk allergens

Food	Target allergen	Heat treatment	IgE binding	Resistance to digestion	References
Milk		95-100 °C 10 min	D		Tiwari and Wolber, 2010
Milk		72°C 15s 100°C 1 min		I	Almaas et al., 2006
Milk		75°C 15s	NE		Host and Samuelsson, 1988
	β -lactoglobulin	100°C 2-10 min 74-100°C 15-60 min	D		Norgaard et al., 1996; Ehn et al., 2004
	β -lactoglobulin	80-100°C 15-120 min	D	D	Morisawa et al., 2009
	β -lactoglobulin	65-95°C 20 min	D		Taheri-Kafrani et al., 2009
	α -casein	80-100°C 15-120 min	NE	NE	Morisawa et al., 2009
	α -casein	82°C 30s 120°C 10 min		I	Dupont et al., 2010

I: increase; D: decrease; NE: no effect.

Table 2. Effect of heat treatment on IgE binding and digestibility of tree nuts.

Tree nut	Target allergen	Heat treatment	IgE binding	Resistance to digestion	References
Walnut		autoclaving, roasting, oil roasting, blanching	NE		Su et al., 2004
Brazil nut	<i>Ber e 1</i>	100°C 20 min; 110-120°C 20 min		NE	Moreno et al., 2005; Van Boxtel et al., 2008b
Pecan		baking; blanching, autoclaving, roasting	NE	D	Venkatachalam et al., 2006
Hazelnut		100-185°C 15-90 min; roasting	D		Wigotzki et al., 2000; Hansen et al., 2003; Worm et al., 2009.
Almond		autoclaving, roasting, oil roasting, blanching	D	D	Bargman et al., 1992; Kong and Singh, 2009
Cashew		autoclaving, roasting, oil roasting, blanching	NE		Su et al., 2004
	<i>Ana 1, Ana 2, Ana 3</i>	autoclaving, roasting, blanching	NE		Venkatachalam et al., 2008
Pistachio		roasting	D	I	Noorbaksh et al., 2010

I: increase; D: decrease; NE: no effect.

Table 3. Effect of different heat treatments on IgE binding of some types of seafood

Seafood	Heat treatment	IgE binding	References
Mackerel	steam sterilisation	D	Chopin et al., 2000
Pomfret	boiling or frying	D	Chatterjee et al., 2006
Hilsha	boiling or frying	D	Chatterjee et al., 2006
Salmon, haddock, mackerel	smoking	I	Sletten et al., 2010
Whiff	boiling	I	Griesmeur et al., 2010
Mackerel	boiling or frying	NE	Chatterjee et al., 2006; Mondal et al., 2007
Bethki	boiling or frying	I	Chatterjee et al., 2006; Mondal et al., 2007

I: increase; D: decrease; NE: no effect.

Table 4. Effect of boiling on IgE binding of some types of shellfish

Shellfish	IgE binding	References
White squid	NE	Yadzir et al., 2010
Shrimp	I	Carnes et al., 2007; Liu et al., 2010
Lobster	I	Carnes et al., 2007
Limpet	I	Carrillo et al., 1994
Razor Shell	I	Martin-Garcia et al., 2007
Abalone	NE	Lopata et al., 1997
Whelk	NE	Lee and Park., 2004

I: increase; D: decrease; NE: no effect.